“Characterisation of phytopathogenic abilities of leaf fungi of common legume crops from Chhattisgarh”

Introduction

The legumes are the large group second only to cereals as a source of human food value, energy, minerals and vitamins in addition to their role in the maintenance of soil fertility. Legumes supply most of the plant proteins which are not only the main but also the cheapest source of dietary proteins. Legume plants are notable for their ability to fix atmospheric nitrogen by symbiotic relationship with bacteria “Rhizobia” found in root nodules of these plants. This ability of mutualism reduced fertilizer costs for farmers and gardeners who use legumes and allow them to be used in a crop rotation to replenish soil. Seeds and foliage of legume crops have comparatively more high protein content to that of non-legume material, due to the additional nitrogen that legumes receive through nitrogen fixation symbiotically. Effective control of crop plant disease is essential to man for making the provision of producing sufficient food for the ever expanding human population. It is also important to maintain the food quality of our crops so that human must not face health problem and also fetch a higher economic return to the producer. The importance of legume in cropping pattern has been stressed by several writers in early Egyptian dynasties and Roman era.

Plant diseases have caused direct economic loss such as the estimated one billion dollars loss in one year. Fungi are the most important group of pathogens and a very wide range of species are parasites of the legume crops, which reduce the crop yields. Species of A*lternaria, Fusarium, Trichothecium and Cladosporium* were associated with poor emergence of soybean in north western hill of Uttar Pradesh (Sharma 1987). The incidence and distribution of *Phytophthora* blight of pigeonpea in different agro climatic zones of Uttar Pradesh (Mishra and Shukla 1987). *Fusarium moniliforme* is a destructive seed borne pathogen of legume crop and cereals and is able to establish seedling blight, wilt, scorch, foot rot, hypertrophy etc. and different seed born fungi like *Aspergillus, Chaetomium, Helminthosporium, Penicillum* etc. obtained from soybean in Nigeria (Popoola and Akueshi 1986). *Fusarium moniliforme* has been reported in *Glycine max* (Nik 1981).

In this piece of research work, 10 crops have been selected, 5 each from Kharif and Rabi crops.

Kharif crops: viz. 1. *Cajanus cajan* (Arhar) 2. *Dolichos lab-lab* (Sem)

3. Glycine max (Soyabean) 4. *Phasiolous mungo* (Mung)

5. *Phasiolous radiatus* (Urad)

Rabi crops: viz. 1. *Lathyrus sativus* (Teora) 2. *Pisum arvense* (Chhota batra)

3. Pisum sativum (Matar) 4. *Lens culinaris* (Massur)

5. Trigonella foenumgraecum (Methi)

These legume crops are commonly cultivated in Chhattisgarh and are consumed considerably by the common masses.

This work includes survey, screening and morphological variations of selected healthy & disease caused leaves, isolation of the fungal pathogens and pathogenicity tests. The isolated pathogen will be tested for their sugar utilization, efficiency, sugar biomass ratio, protein, organic acids and amino acids. The pectolytic and cellulolytic enzyme as well as the intracellular phosphatases will be estimated. The studies will also include both *in-vivo* and *in-vitro* control measures so as to get disease free healthy crops.

A brief review of the work

Pathogenicity of plant pathogens refers to its disease causing ability and various other factors including host-pathogen interactions Brown (1936) defined pathogenesis as "process of disease development include three stages: pre penetration, penetration and post penetration". During disease development pathogenic agents utilize nutrients according to their efficiency and lower the nutritional ability and germination power of the host (Sinha *et al.* 1979, Choudhary *et al*. 1984, Maheshwari *et al*. 1984, Nair 1994, Bindu 1997). Screening programme started in 1960 at the Department of Pathology, College of Agriculture, Parbhani (Zote and Dandnaik, 1987). The molecular basis of disease relations between the host and pathogen influenced, Flor (1942), in proposing a gene for gene hypothesis.

There is also variation in speed of appearance of disease symptoms depending host cultivars (Krishna *et al.* 1987) by their work on powdery mildew in pea. They found that certain cultivars of pea showed slow mildewing of leaves during pathogenesis. Difference in the inoculation period of a pathogen may indicate the difference in host resistance as well as virulence of the pathogens (Mayee and Kokate 1987).

Enzymes are biocatalysts, found in all living cells. They play an important role in pathogenesis. Some extra cellular enzymes like pectolytic, cellulolytic and some enzymes are intra cellular in nature like phosphatase, acid and alkaline phophatases which catalyses hydrolysis of any phophomonoester to give inorganic phosphate are involved in energy metabolism of organism. In several fungi pathogenic to plants have been found to produce them (Zalokar 1960, Naoi *et al*. 1977, Sakurai *et al.* 1977, Arst *et al.* 1980, Rokusu *et al.* 1982, Oleson *et al*. 1981, Arora and Ali 1983). Cellulolytic and pectolytic enzyme in pathogenesis has been reported by many workers (Wood 1960, Janardhanan and Hussain 1969, Goyal and Mahrotra 1980, Bahkali 1989).

Amino acid constitutes the building blocks of all the proteins found in the organic world. Amino acids are classified into alkyl, acidic, imino, hydroxyl, basis sulphur and aromatic groups. The biosynthetic pathways yielding the protein amino acids are well established (Floden 1973). Sugar utilization efficiency is variable in different organisms (Arora 1984, Karkashe 1984). The acid and alkaline phosphateses which catalyses hydrolysis of any phosphomonoester to give inorganic phosphate are involved in the energy metabolism of organisms (Arst *et al.* 1980, Oleson *et al.* 1981, Rokusu *et al.* 1982). Micronutrients are those elements essential for plant growth which are needed for plant growth in very small

quantities. These elements are sometimes called trace elements. The importance of trace elements in the establishment of legumes has been proved in Australia produced 100% increase in yields of certain crops on the semi arid decent trap by applying small dozes of manganese and boron. The latter deficiency causes “Yellows” in lucerne zinc is also important. Investigation of free amino acid content of 20 isolates of *Fusarium solani, f. phaseoli* (Charle’s 1959).

Among the nitrogen sources the mucoraceous organisms have been shown not to utilize the nitrate nitrogen (Casida 1968, Bilgrami and Verma 1981). In a host pathogen relationship, the host must provide an adequate nutritional environment to the infective parasite to permit its potential metabolism and proliferation (Garber 1956). Sulphate sulphur is the general favourite in fungal nutrition (Hawker 1950, Lilly and Barnett 1951, Cochrane 1958). The nutritional composition of mungbean was reported (Singh and Kanungo 1987) and in that of soybean (Eka and Kay 1977). The increase in the nutrient uptake of NPK was found best when VAM inoculum applied at the time of sowing (Mali, Shah and Bhatnagar 2009).

Foliar sprays of Dithane M-45 and Dithane Z-78 for the incidence of angular leaf spot in beans Himanchal Pradesh (Sohi and Sharma 1969, Singh and Sharma 1976, Crowdy 1971). Aureofungin and griseofulvin in the control of angular spot of legumes (Haware and Pavgi 1969). Hystain against dutch-elm disease (Costonis and Davis 1976), streptomycin for control of fireblight (Beer 1976), halo blight of beans (Taylor and Dudley 1977). Dithane Z-78 was the most effective fungicide for *C. linaemuthia* on *Dolichos lab-lab (*Zote *et al.* 1976). Several follicolous pathogens of soybean including the *C.truncatum* (Almedia 1981). Bavistin and Bavistin + brassicol with 3 spray of bavistin were the next best treatment for the control of Ascochyta blight of *Chickpea* (Tripathi, Singh and Choube 1987). Application of multi-strain compost carriers fortified with biocontrol and biofertilizer against in a field trail with some legume plants significantly reduced wilt disease caused by *Fusarium oxysporum* and increased plant dry weight, plant height and yield (Wafaa, Haggag and Saber 2000).

Objectives

1. Survey & screening of diseased leaves of selected crops from different fields
2. Isolation of fungal pathogens
3. Pathogenicity test
4. Growth and cultural characteristics on the basis of
    A. Selection of basal media
    B. Sugar biomass ratio
5. Biochemical analysis of isolated pathogens of
    A. Protein (by Lowry's method)
    B. Chromatographic analysis of
        a. Amino acids
        b. Organic acids
        c. Sugars
6. Nutritional analysis
    A. Mineral composition
    B. Trace elements
7. Enzymological studies
    A. Extracellular enzymes
        a. Cellulolytic
        b. Pectolytic
    B. Intra cellular enzymes

    Phosphatases
        a. Acid phosphatase
        b. Alkaline phosphatase
8. Disease protection (*in-vivo, in-vitro*)
    A. Antibiotics
    B. Fungicides
    C. Angiospermic sources

Noteworthy contribution in the field of proposed work

The age of the host (Soyabean) plant at the time of inoculation significantly influenced the development of Myrothecium leaf spot disease was studied (Shrivasatava and Khan 1993). Pectic enzyme of the pathogen were instrumental in the chocolate spot of beans have been found (Deverall and Wood 1961). The biosynthetic pathways yielding the protein amino acids are well established (Floden 1973). Study on amino acid fermentation by quite a large number of microorganisms, observed that almost all the organisms screened gave a positive indication of amino acid(s) (Kinoshita *et al.* 1957, 1958). Enormous protein production capacity in *Fusarium solani* was studied (Mangatt 1989). Contribution of legumes to diet is considerable in terms of protein. Chemical composition, protein, digestibility and amino acids in mungbean genotype have been reported (Rao *et al.* 1978). Knowledge of nutritional requirement of parasitic fungi was stated (Hurley 1936). The fact that physical environment influences nutritional demands of microorganism is well recognized (Lichstein 1960). Percent content in uptake of nutrient (NPK) was higher in soybean plants inoculated with VAM fungi (Mali, Shah and Bhatnagar 2009). Extract of ginger rhizome played a significant role in control of powdery mildew in pea plants (Singh *et al.* 1991). A significant negative effect of foliar spray of potash and neem seed extract on tikka leaf spot of groundnut (Chandrashekhar *et al.*1993). Economical and effective control measures through the use of chemicals as foliar sprays (Sharma 1987). Leaf extract of marigold (*Tagetes erecta*) follwed by neem extract (*Azadirechta indica*) caused maximum inhibition of mycelial growth and sclerotial production its size and viability (Singh *et al.* 2007).

Proposed Methodology

Survey, Screening & Isolation

1. For the present studies the fungi will be collected from 5 different sites including urban & rural area. 10 legume crops will be selected for isolation of leaf spot diseases, five each from "Kharif" and five each from "Rabi".

   Kharif crops: viz. 1. *Cajanus cajan* (Arhar) 2. *Dolichos lab-lab* (Sem)

   3. *Glycine max* (Soyabean) 4. *Phasiolous mungo* (Mung)

   5. *Phasiolous radiatus* (Urad)

   Rabi Crops: viz. 1. *Lathyrus sativus* (Teora) 2. *Pisum arvense* (Chhota batra)

   3. *Pisum sativum* (Matar) 4. *Lens culinaris* (Massur)

   5. *Trigonella foenumgraecum* (Methi)

2. The fungal pathogens from these samples will be isolated according to the standard methods.

3. Pathogenicity will be confirmed by Koch's postulates.

4. Pure cultures of the isolated pathogens will be maintained for further studies.

   To find out the cultural characteristics of the fungal pathogens the parameters like selection of basal medium, sugar biomass ratio, nutritional characteristics of the fungal pathogens will be assessed.

   For analysis of biochemical characteristics of protein, (Lowry's method) chromatographic analysis of amino acids, organic acids, sugars, mineral composition and trace elements will be done.

   Enzymes are considered to be basically responsible for pathogenesis. Certain extra cellular enzymes like pectolytic and cellulolytic will be assayed using standard method (Bateman 1963, Gascoigne 1960, Hancock 1964). Intracellular enzyme like acid and alkaline phosphatase will be assayed spectrophotometrically. Control measures will be applied to check diseases *in-vitro* as well as *in-vivo* using, for which several controlling agents like antibiotics, fungicides and angiospermic sources will be tested.

Expected Out Come

This piece of research shall contribute in:-

1. Survey, screening and isolation of follicolous necrotic fungi from some common Kharif & Rabi crops of Chhattisgarh shall definitely contribute in the collection of base line data of legume plant diseases.
2. Pathogencity test, growth and cultural characteristics will certainly manifest the disease incidence and shall be helpful in deriving out the host pathogen relationships.
3. Biochemical analysis of fungal pathogens, their nutritional assessment and role of enzymes will help to assess the process of disease development.
4. Disease protection with the help of antibiotics, fungicides and angiospermic sources shall aid to get healthy and disease free crops in Chhattisgarh.

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